ELECTRON MICROSCOPIC STUDY OF THE MORPHOLOGICAL CHANGES IN INTESTINAL BACTERIA AND ANTHRACOIDS FOLLOWING THE ACTION OF SOME PHENOL PREPARATIONS

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ELECTRON MICROSCOPIC STUDY OF THE MORPHOLOGICAL CHANGES IN INTESTINAL BACTERIA AND ANTHRACOIDS FOLLOWING THE ACTION OF SOME PHENOL PREPARATIONS

[Following is the translation of an article by Ye. K. Skvortsova and N. P. Likhacheva, Central Scientific-Research Disinfectant Institute, published in the Russian-language periodical Zhurnal mikrobiologii, Epidemiologii i immunobiologii (Journal of Microbiology, Epidemiology, and Immunobiology), Jan 1963, V.40,pp 88-91. It was submitted on 22 Aug 1961. Translation performed by Sp/6 Charles T. Ostertag Jr.]

The study of the morphological changes which take place in microorganisms under the influence of disinfectants is an important step in the perception of the mechanism of action of the latter. From an analysis of works which have been published on this problem (Pekhov, 1956; Ivashkevich et al., 1959; Med, 1943; Minami, 1957; Salton and Horne, 1951) it follows that the reaction of the microbial cell to the actions of various harmful agents may be diverse.

In the present work, with the aim of clearing up the mechanism of action of disinfectants, we have studied the morphological changes which take place in a microbial cell under the influence of disinfectants of the phenol group. The bactericidal action of phenol and its derivatives is generally well known. Among the substances of this group there are preparations which show a very strong bactericidal effect (hexylresorcin and heptylresorcin) along with preparations possessing a comparatively low bactericidal capability (phenol, resorcin). In our investigations we used both these and other preparations.

A suspension in distilled water of a 24-hour culture of intestinal bacteria or anthracoides containing 2 billion microbial cells in 1 ml was placed in a solution of disinfectant. The mixture was centrifuged for 10 minutes at 3,000 rpm's. The sediment was washed off twice and after resuspension was deposited on a collodion film. The preparations were contrasted with chromium vapors. Observations and photographing were carried out in an EM-3 electron microscope under an accelerated voltage of 50 kilovolts.

In order to establish the dynamics of the morphological changes of the microorganisms, the actions were studied of both bactericidal and subbactericidal solutions.

On the electron micrograms, the young forms of intestinal bacteria were homogeneous. They had a uniform electron-optical density. Upon aging there appeared in the cell electron-optical dense granules, arranged with one or two at each pole or along the longitudinal axis of the microbial cell (fig 1a).

Following the action of a 1.5% solution of phenol the homogeneous protoplasm of the cell took on a coarse-grained appearance, crumpled up, and withdrew from the inner side of the cell wall which appeared unharmed.

Increasing the concentration of the phenol solution up to 2% caused a sharper coagulation of the protoplasm. In some of the specimens the cell walls broke down and the contents of the cells escaped into the surrounding medium in the form of dense cytoplasmatic granules. The cellular membrane, being empty, maintained the form of the bacterial cell (fig 1,b).

The action of subbactericidal 2% solutions of resorcin didn't caused distinct morphological changes. In the protoplasm the appearance was noted of a barely noticeable finely divided granularity which was intensified when the concentration of the solution was increased to bactericidal. Owing to coagulation during this a fine granularity appeared in the protoplasm. The cell wall remained unharmed and in the form of a thin stria framed the shriveled protoplasm.

Subbactericidal 0.025% solutions of hexylresorcin caused changes which led to a considerable polymorphism in the structure of the intestinal bacteria. We treated these changes as various stages of one and the same process. Increases in sizes were observed superficially as if swollen from an increase in the intracellular osmotic pressure of the cell. The presence of shadows, which appeared after shadow casting with chromium, testified to the increase of the volumetric depth of these cells. But such cells were few. Often specimens were noted with the entirety of the cell wall disrupted and with a clearly expressed escaping of the contents of the cell (fig 1,v). Preferably this disruption can be called disruption of the permeability of the cell wall, as a result of which the protoplasm flowed out uniformly throughout the entire surface of the cell. The cell walls which were freed of protoplasm had well defined contours and based on their lowered electron-optical density were different from the cell walls which were observed following the action of phenol.

Degenerative changes in the cells of intestinal bacteria following the action of hexylresorcin were also expressed in the coagulation of protoplasm - the appearance of a compact grain structure with minor contractions. The bactericidal effect of 0.05% solutions of hexylresorcin was accompanied by sharp changes of the protoplasm which had the nature of a fine-grained coagulation with granules of a high electron-optical density.

The action of solutions of heptylresorcin both in subbactericidal (0.025%) as well as bactericidal (0.05%) concentration was manifested in changes of the protoplasm. The sharpest destructive changes set in following the action of the higher concentration of the preparation. The protoplasm was contracted into a coarse-grained mass with a high electron-optical density. The cell wall wasn't harmed (fig 1,g).

The anthracoides on electron micrograms had a high electron-optical density which didn't permit the observation of any kind of structural peculiarities of the bacillus. Only the flagella were well discernible (fig 2,a). After the action of a 3% solution of phenol (lower concentrations didn't cause any morphological changes), a sharp coagulation of the protoplasm was observed and the cells decreased somewhat in volume. The most natural feature characterizing the action of phenol was the appearance in the protoplasm of sections with a lowered penetrability for electrons.

The action of a 4% solution of resorcin on anthracoides was expressed mainly in changes of the electron-optical density. The cell was as if broken up, its protoplasm consisted of grains of various size and electron-optical density, the cell wall was discernible (fig 2,b), and individual cells broke up into fine protoplasmatic grains with a vague form. The entire mass which was breaking up maintained the form of the bacterial cell.

Other morphological changes were observed following the action of a 2% solution of hexylresorcin. The cells appeared to be sharply increased in volume, the wholeness of the cell wall was disrupted and the intracellular contents were escaping into the surrounding medium in the form of large grains. The electron-optical density was sharply reduced and at the same time was identical throughout the extent of the entire mass of the cell (fig 2,v). Along with this, cells were met in the preparations which didn't have any distinct morphological changes.

Heptylresorcin in the form of a 2% solution didn't exert a similar effect on the anthracoides. The protoplasm remained homogeneous. Only in individual cells was there a considerable reduction of electron-optical density and consequently in these places the cell was somewhat expanded in girth.

Thus in accordance with our observations, phenol preparations caused diverse morphological changes in microorganisms which excluded the uniformity of actions even of substances which are related by their chemical structure. The phenomenon of bacteriolysis in intestinal bacilli after the action of hexylresorcin was sharply different from the breaking up of the bacterial cell after its contact with phenol. Posthumous changes of the cells after the action of disinfectants, based on the electron microscopic picture, didn't conform to their autolytic break down following aging and natural necrosis.

Resorcin and heptylresorcin, under the conditions of the experiment conducted, didn't cause a bacteriolytic effect, and the bactericidal effect in regards to the intestinal bacillus was expressed in destructive changes of the plasma content which were manifested more sharply following the action of heptylresorcin.

Various cytological reactions were observed following the brief treatment of anthracoides with the same phenol substances. Changes observed in the structure of anthracoides following the action of each preparation were diverse and had nothing in common with changes of an autolytic nature in aging cells.

The action of one and the same substance - hexylresorcin (just as with other substances) - on different species of microorganisms caused a different picture of morphological changes of the cells which, in all probability, can be explained by the morphological peculiarities of the species of microorganisms. As a general feature in the action of all preparations can be considered the appearance of destructive changes of the protoplasm and as a consequence of the latter, changes of the electron-optical density of the bacterial cell. A bacteriolytic effect in the intestinal bacillus was observed following the action of hexylresorcin and phenol, and in anthracoides - following the action of resorcin and hexylresorcin.

Considering the diverse morphological changes in the bacterial cell it can be assumed that the courses of action of the substances under study on the vitally important processes taking place in the microbial cell are various and can speak for the specificity of the reactions of the microorganisms to the action of the various chemical agents.

CONCLUSIONS

1. Phenol, resorcin, hexylresorcin and heptylresorcin caused various morphological changes in intestinal bacilli and anthracoides.

2. The morphological changes of the bacterial cell following the action of the specified compounds were determined to a known level by the morphological peculiarities of the species of microorganisms.

Figure 1 (page 89) Intestinal bacillus

a - initial 24-hour culture; b - following the action of a 1.5% solution of phenol; v - following the action of a 0.025% solution of hexylresorcin; g - following the action of a 0.05% solution of heptylresorcin. Magnification 14,000 x 2.

Figure 2 (page 90) Anthracoides

a - initial 24-hour culture; b - following the action of a 4% solution of resorcin; v - following the action of a 2% solution of hexylresorcin. Magnification - 10,000 x 2.

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[Following is the English summary which appears with the Russian article.]

In electron microscopic study of morphological changes in E. Coli and Bact.anthracoides after the action of phenol preparations (phenol, resorcin, hexylresorcin and heptylresorcin) it was established that each of these preparations caused various disturbances of bacterial cell structure, also determined by the morphological peculiarities of the microorganism species.